Customized FORM PTO-1390 U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY DOCKET NO
P07109US00/BAS

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)
09/787195

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB99/03109	17 SEPTEMBER 1998	17 SEPTEMBER 1998

TITLE OF INVENTION: BIO-ASSAY TECHNIQUE

APPLICANT(S) FOR DO/EO/US: DAMES, Andrew et al.

Applicant herewith submits to the US Designated/Elected Office (DO/EO/US) the following items and other information:

☒ 1. This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

☐ 2. This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 USC 371.

☒ 3. This express request to begin national examination procedures (35 USC 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 USC 371(b) and PCT Art. 22 and 39(1).

☒ 4. A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

☒ 5. A **copy** of the International Application as filed (35 U.S.C. 371 (c)(2))
- ☐ a. is transmitted herewith (required only if not transmitted by the International Bureau).
- ☒ b. has been transmitted by the International Bureau.
- ☐ c. is not required, as the application was filed in the United States Receiving Office (RO/US).

☐ 6. A **translation** of the International Application into English (35 U.S.C. 371(c)(2)).

☒ 7. Amendments to the claims of the International Appln. under PCT Article 19 (35 USC 371 (c)(3))
- ☐ a. are transmitted herewith (required only if not transmitted by the International Bureau).
- ☐ b. have been transmitted by the International Bureau.
- ☐ c. have not been made; however, the time limit for making such amendments had NOT expired.
- ☒ d. have not been made and will not be made.

☐ 8. A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

☐ 9. An **oath** or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

☐ 10. A translation of the annexes to the Int'l Prelim. Exam. Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

☐ 11. An **Information Disclosure Statement** under 37 C.F.R. 1.97 and 1.98.

☐ 12. An **Assignment** document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

☒ 13. A **First preliminary amendment**.

☐ A Second or subsequent preliminary amendment.

☐ 14. A substitute specification.

☐ 15. A change of power of attorney and/or address letter.

☐ 16. Other items or information:

☐

☐

☐ A copy of the Notification of Missing Requirements under 35 U.S.C. 371.

☐ In the event that a petition for extension of time is required to be submitted herewith, and in the event that a separate petition does not accompany this response, applicant hereby petitions under 37 CFR 1.136(a) for an extension of time of as many months as are required to render this submission timely. Any fee is authorized in 17(c).

Date: 15 MARCH 2001

U.S. APPLICATION NO. (If known) 09/787195	INTERNATIONAL APPLICATION NO. PCT/GB99/03109	ATTORNEY DOCKET NO. P07109US00/BAS

	CALCULATIONS PTO USE ONLY
☒ 17. **The following fees are submitted:**	
☒ **Basic National Fee** (37 CFR 1.492 (a) (1)-(5):	
☐ Neither Int'l Prelim. Exam. fee nor Int'l Search fee paid to USPTO $1000	
☒ Search Report has been prepared by the EPO or JPO $ 860	
☐ No Int'l Prelim. Ex. fee paid to USPTO but Int'l Search fee paid to USPTO $ 710	
☐ International preliminary examination fee paid to USPTPO $ 690	
☐ Int'l Prelim. Ex. fee paid to USPTO & all claims satisfied PCT Art. 33(1)-(4) $ 100	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860
☐ Surcharge of **$130** for furnishing the oath or declaration later than ☐ 20 mos. ☐ 30 mos. + from the earliest claimed priority date (37 CFR 1.492(e)).	$

CLAIMS	*NUMBER FILED*	*NUMBER EXTRA*	*RATE*	
Total Claims	21 - 20 =	1	X **$18** =	$ 18
Independent Claims	1 - 03 =		X **$80** =	$
☐ Multiple Dependent Claim(s) (if applicable)			+ **$270** =	$
			TOTAL OF ABOVE CALCULATIONS =	$ 878
☒ Reduction of ½ for **small** entity status of applicant.				$ 439
			SUBTOTAL =	$ 439
☐ Processing fee of **$130** for furnishing the **English translation** later than ☐ 20 mos. ☐ 30 mos. + from the earliest claimed priority date (37 CFR 1.492(f)).				$
			TOTAL NATIONAL FEE =	$ 439
☐ Fee for recording the enclosed **assignment**, accompanied by a cover sheet - **$40** per property				$
			TOTAL FEES ENCLOSED =	$ 439
			Amount to be *Refunded*	$
			Amount to be *Charged*	$

☒ a. A **check** in the amount of $439 to cover the above fees is enclosed.

☐ b. Please charge my Deposit Account No. 12-0555 in the amount of $ to cover the above fees.

☒ c. The Commissioner is hereby authorized to charge any additional fees required or credit overpayment to Deposit Account No. 12-0555.

Note: *Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.*

SEND ALL CORRESPONDENCE TO:

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At the address (below) of **CUSTOMER NO. 00881.**

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REG. NO.: 28518

PHONE NO.: 703-739-4900

Date: 15 March 2001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent

In re patent application of: DAMES et al.

Serial No.: New Application — Examiner:

Filed: On even date herewith — Art Unit:

For: BIO-ASSA TECHNIQUE — Docket No.: P07109US00

AMENDMENT

Assistant Commissioner for Patents
Washington, D.C.

S I R:

Preliminary to the examination thereof, please amend the above-identified application as follows:

IN THE CLAIMS

A clean version of all pending amended claims is provided herewith in **Attachment A**. It will be noted that claims 1-5, 7,10-12,14, 16 and 20 have been amended relative to the previously provided version as shown by the marked up version thereof in **Attachment B** provided herewith.

REMARKS

The above amendments to claims have been made in order place the application in better condition for examination and for the purpose of reducing the filing fees.

Respectfully submitted,

Date: 3/15/01

By: Douglas E. Jackson
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ATTACHMENT B

Marked Up Replacement Claims

Following herewith is a marked up copy of each rewritten claim together with all other pending claims.

1. (amended) A solid support ~~(1,22)~~ for a biochemical assay, which support is substantially linear or planar in shape and has an anodised metal surface layer ~~(13)~~, the largest dimension of the support being less than 100μm, whereby an aqueous suspension is formable from a plurality of the supports.

2. (amended) A support according to claim 1, wherein the surface layer has cellular structure anodisation layer ~~(15, 23)~~, the growth direction of the cells of the anodisation layer being perpendicular to the plane of the surface layer.

3. (amended) A support according to claim 1 ~~or 2~~, wherein probe molecules ~~(16)~~ for the biochemical assay are bound to the surface layer.

4. (amended) A support according to ~~any one of claims 1 to 3~~ claim 1, wherein the surface layer is of aluminum.

5. (amended) A support according to ~~any one of claims 1 to 4~~claim 1, wherein the surface layer is porous.

6. A support according to claim 5, wherein the pore size of the surface layer is approximately matched to the biochemically active molecules to be bound.

7. (amended) A support according to ~~any one of claims 1 to 6~~claim 1, incorporating a spatially varying pattern ~~(18)~~ for identification purposes.

8. A support according to claim 7, wherein said pattern is a barcode.

9. A support according to claim 8, wherein the barcode is a linear barcode.

10. (amended) A support according to ~~any of claims 7 to 9~~claim 1, in which the pattern comprises a series of holes ~~(2)~~ in the support.

11. (amended) A method of fabricating the supports of ~~one of claims 1 to 10~~claim 1, comprising sputter coating a flat surface with metal layer ~~(13)~~, anodising the metal layer, and lithographically patterning and etching the metal layer to reveal the supports.

12. (amended) A method according to claim 13, wherein said surface consists of layer of soluble material ~~(12)~~ on a rigid substrate ~~(11)~~, and the method further comprises releasing the supports from said surface by solvation of the soluble material.

13. A method according to claim 12, wherein the soluble material is a resist.

14. (amended) A method according to ~~any of claims 11 to 13~~claim 11, wherein the anodising is carried out at a voltage of up to 150 V.

15. A method according to claim 14, wherein the anodising is carried out at a voltage in the range from 4 V to 30V.

16. (amended) A method according to ~~any one of claims 11 to 15~~claim 11, further comprising binding probe molecules ~~(16)~~ to the anodised metal layer.

17. A optical reader for reading the patterns and identifying the supports according to claim 7.

18. A reader according to claim 17, operating by means of transmission optics.

19. A reader according to claim 18, wherein said supports are transported through an optical read volume by a fluidic system.

20. (amended) A reader to claim 18, in which there are two substantially orthogonal light transmission paths ~~(33, 34)~~.

21. A reader according to claim 20, incorporating one or more fluorescence detectors.

ATTACHMENT A

Clean Replacement/New Claims (entire set of pending claims)

Following herewith is a clean copy of the entire set of pending claims.

1. (amended) A solid support for a biochemical assay, which support is substantially linear or planar in shape and has an anodised metal surface layer, the largest dimension of the support being less than 100μm, whereby an aqueous suspension is formable from a plurality of the supports.

2. (amended) A support according to claim 1, wherein the surface layer has cellular structure anodisation layer, the growth direction of the cells of the anodisation layer being perpendicular to the plane of the surface layer.

3. (amended) A support according to claim 1, wherein probe molecules for the biochemical assay are bound to the surface layer.

4. (amended) A support according to claim 1, wherein the surface layer is of aluminum.

5. (amended) A support according to claim 1, wherein the surface layer is porous.

6. A support according to claim 5, wherein the pore size of the surface layer is approximately matched to the biochemically active molecules to be bound.

7. (amended) A support according to claim 1, incorporating a spatially varying pattern for identification purposes.

8. A support according to claim 7, wherein said pattern is a barcode.

9. A support according to claim 8, wherein the barcode is a linear barcode.

10. (amended) A support according to claim 1, in which the pattern comprises a series of holes in the support.

11. (amended) A method of fabricating the supports of claim 1, comprising sputter coating a flat surface with metal layer, anodising the metal layer, and lithographically patterning and etching the metal layer to reveal the supports.

12. (amended) A method according to claim 13, wherein said surface consists of layer of soluble material on a rigid substrate, and the method further comprises releasing the supports from said surface by solvation of the soluble material.

13. A method according to claim 12, wherein the soluble material is a resist.

14. (amended) A method according to claim 11, wherein the anodising is carried out at a voltage of up to 150 V.

15. A method according to claim 14, wherein the anodising is carried out at a voltage in the range from 4 V to 30V.

16. (amended) A method according to claim 11, further comprising binding probe molecules to the anodised metal layer.

17. A optical reader for reading the patterns and identifying the supports according to claim 7.

18. A reader according to claim 17, operating by means of transmission optics.

19. A reader according to claim 18, wherein said supports are transported through an optical read volume by a fluidic system.

20. (amended) A reader to claim 18, in which there are two substantially orthogonal light transmission paths.

21. A reader according to claim 20, incorporating one or more fluorescence detectors.

Bio-assay Technique

Field of the Invention

This invention relates to the field of micromachined or microfabricated coded substrates, particularly but not exclusively for use as a parallel bioassay technique.

Background

Massively parallel bioassay tests are the enabling techniques that have made the majority of recent advances in genetics, screening and drug discovery possible. Thousands or millions of tests that previously were carried out one by one may now be combined into a single experiment yielding thousands or millions of results. The key to this process has been the development of techniques that allow the results of the many different tests to be separated from each other.

A number of existing techniques are described below. The techniques consist of labelling each of the constituent experiments in a manner that can be read after the experiment has completed. Labels used at present include the position of the experiment on the surface of a test chip and the fluorescent spectrum of a particle to which the experiment is bound.

Affymetrix's GeneChip probe array is a DNA sequence testing chip, where tens of thousands of different DNA probes are located at known points on a large 2D array. The fabrication process is described in, for example US 5,744,305 or US 5,143,854. Standard DNA hybridisation techniques are used in a chamber above the chip, and the test results are read out optically by the positions of fluorescent dots on the array (see, for example, US 5,578,832). The combinations of tests are pre-determined during the manufacture of the chip.

Luminex Corporation's FlowMetrix system makes use of coded microspheres, 6.5 μm in diameter. Each bead incorporates red and orange fluorophores to make up the code. Eight different intensities are possible, allowing 64 different bead types. A green fluorescent marker is used for the probes. This system is described in US 5,736,330. The system has a relatively small number of codes, and requires complex, multi-wavelength optics on flow cytometers to read the codes and fluorescence of the test.

NanoGen has a semiconductor-based microchip array, APEX, which is aimed specifically at DNA binding and sequencing experiments. NanoGen's chip is programmable by the end user with different arrays of DNA probes (see, for example, US 5,605,662 and US 5,929,208).

There are a number of other particle or substrate-based assay techniques under the general heading of Combinatorial Chemistry. For examples, WO 96/24061 describes a library of tests using radio-frequency identification tags. WO 97/32892 describes a composite support for use a combinatorial chemistry substrate. GB 2306,484 describes two-part support particles for combinatorial chemistry.

Summary of the Invention

This invention describes a system for carrying out massively parallel multiple bioassay tests in a low-cost, fast and convenient manner. The scheme involves making a suspension (an assay) containing many thousands of different types of micro-machined coded labels, (micro-labels), each code carrying a different biochemical test or probe.

An assay is constructed by mixing together suspensions of chosen sets of active micro-labels. Assays are customised to particular applications, independently of the original fabrication of the micro-labels.

The sample under test is marked with a fluorescent label and incubated with the assay. Only micro-labels with probes that bind to the fluorescent sample molecules will become fluorescent.

The micro-labels are fabricated from an anodisable material such as aluminium, initially deposited onto a planar substrate with a soluble release layer. The metal surface is anodised before patterning. This allows the attachment of a wide range of biochemically active agents for use as highly selective probes.

Standard optical lithography and dry etching is used to pattern the aluminium into separate micro-labels. The code is stored on the micro-labels as a series of holes using coding

schemes similar to those found on bar codes. The biochemical probes may be attached to the surface either before or after the lithography step. The micro-labels are then released into an aqueous suspension. Each different micro-label code has a unique probe associated with it. Micro-labels with up to 100,000 different variants have been demonstrated.

A flow-based reader system, similar to a flow cytometer, draws through thousands of micro-labels per second, reading both the bar code and the result of the test. The test result is measured by the degree of fluorescence. An alternative planar reading system, in which the micro-labels are plated out onto a flat substrate, uses fluorescence microscopy and image processing to read the results of the tests.

Brief Description of the Drawings

Embodiments of the invention will now be described, by way of example, with reference to the accompanying drawings, in which:

Figure 1 illustrates a single micro-label incorporating a transmission optical barcode,
Figure 2 illustrates a parallel bio-assay comprising two binding event experiments,
Figure 3 illustrates a wafer scale fabrication process for micro-labels,
Figure 4 illustrates a porous structure resulting from anodic oxidisation of an aluminium surface,
Figure 5 illustrates a flow through micro-label reading device,
Figure 6 illustrates the use of orthogonal interrogation beams to enable labels to be read regardless of rotation

Detailed Description

Micro-labels

Figure 1 shows a micro-label, 1, in the form of a micro-machined miniature optical bar code, made from aluminium. The bar code is formed by a series of holes, 2, in the aluminium.

Each micro-label of this type is about 100 μm long, 3, by 10 μm wide, 4, by 1 μm thick, 5, and is capable of storing up to 100,000 different codes. Around 10 million such micro-

labels can be fabricated on a single 6-inch diameter substrate. Different lengths of micro-label, from 40-100 μm, carrying between 2 and 5 decimal digits of data, have been fabricated. Each different code is associated with a unique biochemical probe. Coding schemes such as those used in EAN and UPC bar codes are used, to provide strong error checking when the codes are read.

Probes and Assays

With reference to Figure 2, an assay, 6, of micro-labels, is constructed by mixing together suspensions of chosen sets of active micro-labels. Each different code has a unique biochemical probe associated with it, which binds to a specific type of molecule. Binding reactions may be selected from the group consisting of antibody-antigen, enzyme-substrate, enzyme-receptor, toxin-receptor, protein-protein and avidin-biotin.

The sample under test, 8, is marked with a fluorescent label and incubated with the assay. Only micro-labels, 7, with probes that bind to the fluorescent sample molecules will become fluorescent, 10. The result of the test is measured by the degree of fluorescence of different types of micro-labels.

An example of an application of the present invention is the screening of serum for a selection of specific antibodies in a parallel bioassay. Antigens to the antibodies are used as probes and bound to micro-labels marked to identify these antigens. The micro-labels are incubated with the sample and then incubated with an antibody specific fluorescent label. The micro-labels are then passed though a reader that measures the degree of fluorescence of the labels and their identity. Correlation of the identities of the micro-labels with their degree of fluorescence provides indication of the binding of antibodies in the sample with the surface bound antigens.

Micro-label Fabrication

The fabrication process for the micro-labels will now be described with reference to Figure 3.

A substrate material, 11, such as a silicon wafer, is first coated with a soluble release layer, 12. In the preferred embodiment, this is a spin-coated layer of polymethyl methacrylate resist, baked at 150°C to drive off the solvents.

A layer of aluminium, 13, 1 μm thick, is deposited onto the substrate. This is achieved using a standard vacuum sputter coating technique, commonly used in semiconductor device fabrication.

The aluminium layer, 13, is anodised, 14, at a voltage of 30V in 4% phosphoric acid, using a pure aluminium cathode, for 30s, at a current density of 10 mA/cm^2. This leads to a 100 nm anodised layer, 15, with a pore size of around 40 nm.

At this stage (depending on the application) the aluminium surface may be coated with probe molecules, 16, by immersing the surface in an aqueous solution/suspension of the molecules.

The substrate is spin-coated with conventional optical resist, 17, Shipley S1813. The label pattern, 18, is exposed using a hard-contact optical mask, and developed using aqueous alkaline developer (MF319). The pattern is transferred into the aluminium layer using reactive ion etching with $SiCl_4$.

If the probe attachment is to be carried out after the lithography, the optical resist, 17, may be removed at this stage using a solvent such as isopropyl alcohol. This leaves the release layer, 13, intact, with the patterned aluminium layer, 20, on top. The probe molecules, 16, may then be attached as described above, whilst the micro-labels are still attached to the substrate.

The micro-labels with attached probes are released, 21, from the substrate using a solvent such as acetone. Dilution and filtration leaves the micro-labels, 22, in an aqueous suspension ready for combination into an assay.

The fabrication process is suitable for a wide variety of shapes of micro-labels with holes in, including both the substantially linear type of Figure 1 and other forms of square, rectangular and round micro-labels.

Anodisation and Surface Chemistry

Proteins bind only weakly with an untreated aluminium surface when incubated in an aqueous solution. By modifying the surface this binding can be selectively enhanced to control when the binding occurs. This is important because it allows the probe molecules to be bound strongly to the surface at time of manufacture whilst maintaining weak non-specific binding of the fluorescent target molecules during the test. In this way the discrimination of the test is maximised.

There exists a very wide body of knowledge, gained in the first half of this century, on surface protection of aluminium components through the use of electrochemical processes. Methods for growing porous surfaces are well known, as are processes for sealing these surfaces. We have exploited this knowledge to develop a relatively simple process that grows an adsorbing surface with well controlled porosity and depth. This surface binds the chosen proteins well soon after treatment, but heals with time to prevent further binding.

In order to generate the appropriate anodised surface morphology, it is necessary to understand the structure of typical of biomolecules used as probes. Cryogenic atomic-force-microscopy allows direct imaging of such biomolecules. For example, Immunoglobulin-G (IgG) has a Y-shaped structure. The size of the IgG molecule is approximately 40nm across, which can be considered to be a typical size.

With reference to Figure 4, during anodisation, an aluminium oxide layer, 23, grows on the surface of the aluminium. This oxide layer grows in a two dimensional hexagonal cell structure, 24, the size of each cell depending on the electrochemical forming voltage. In the centre of each cell, a pore, 25, forms with an aperture smaller than the width of the cell, 26. As the anodisation process progresses, the thickness, 27, of the oxide layer and the depth of the pores, 28, increase, but the cell width, 26, and the pore aperture, 28, remains constant.

Reading Systems

Two classes of reading system have been developed. These are based on flow cells, and on planar imaging of plated-out micro-labels.

The flow-cell reader, shown schematically in Figure 5, utilises a design that induces the micro-labels, 1, to flow down the centre of a tube, 29 through a defined reading zone, 30. Normally, elongated particles in flow would have a tendency to tumble. However, by using an accelerating sheath fluid, 31, and injecting the micro-labels into the centre of this flow, 32, a hydrodynamic focussing effect is achieved that causes all the micro-labels to align, and pass through a well-defined focal point, 30, somewhere downstream of the injection point, 32.

At the hydrodynamic focal point, 30, two orthogonal focused beams, 33,34, of laser light from a 488 nm argon-ion laser interrogate the micro-labels. This is detailed in Figure 6. The use of two orthogonal interrogation beams enables the interrogation of micro-labels, regardless of their rotation with respect to the frame of reference of the flow tube. The label, 1, in Figure 6, is shown at an angle of 45° to both incident beams, which is the worst possible case. The geometry of the micro-labels, shown in Figure 1, means that light is still transmitted through the holes in the label, 2. The holes modulate the transmitted intensity at the detector, 35, as the label passes through, generating a serial stream of information that is analysed in the same way as a conventional bar code. Simultaneously, the degree of fluorescence is measured, using an epi-fluorescence detector, 36. This is correlated with the code on the label. High numerical-aperture optics (microscope objective lenses) are used to achieve the desired resolution. The optimal flow-cell design has flat walls, to avoid the use of custom cylindrical imaging elements.

Once sufficient micro-labels have been read the reader calculates the results of all the tests. This number required is typically between 10 and 100 copies of each type of micro-label, to enable statistical analysis to be used. The test results show the mean and standard deviation of the fluorescence for each type of probe.

In a planar reading system, the micro-labels are plated out onto a flat substrate. This is either a filter substrate, or a transparent substrate. Conventional fluorescence microscopy

is used to analyse the plated substrate systematically. An image-processing system captures pictures of each field of the substrate. Transmitted light is analysed separately from fluorescent light. Each micro-label is identified from its transmitted light profile, and the fluorescence signal integrated over the surface of the label is recorded. Once again, between 10 and 100 of each type of micro-label are required to give a good statistical analysis.

Applications

A typical application uses a suspension of probes for a number of different antibodies (e.g. hepatitis, HIV). A reading system takes a drop of blood from a patient, labels it with fluorescent marker, and incubates it with the probe suspension for a few minutes. The suspension is fed through a microfluidic detector system.

Further Embodiments

Micro-label designs for planar reading systems are not limited to the linear designs described above, which are primarily intended for flow-through reading systems. Planar systems can use 2D patterns, fabricated on labels which are closer to being square or rectangular, rather than linear. Coding schemes similar to conventional 2D barcodes are then used. The outline of the label also gives information, particularly about orientation.

Labels incorporating magnetic material can use magnetic separation. This is useful if the sample being tested contains solid matter of similar size to the labels that could contaminate the results.

Micro-labels can be fabricated using any substrate that can be coated with an anodisable metal such as aluminium. This is particularly attractive when a mass-production method such as embossing of an aluminium-coated plastic is used to fabricate the micro-labels.

CLAIMS:-

1. A solid support (1, 22) for a biochemical assay, which support is substantially linear or planar in shape and has an anodised metal surface layer (13), the largest dimension of the support being less than 100μm, whereby an aqueous suspension is formable from a plurality of the supports.

2. A support according to claim 1, wherein the surface layer has a cellular structure anodisation layer (15, 23), the growth direction of the cells of the anodisation layer being perpendicular to the plane of the surface layer.

3. A support according to claim 1 or 2, wherein probe molecules (16) for the biochemical assay are bound to the surface layer.

4. A support according to any one of claims 1 to 3, wherein the surface layer is of aluminium.

5. A support according to any one of claims 1 to 4, wherein the surface layer is porous.

6. A support according to claim 5, wherein the pore size of the surface layer is approximately matched to the

size of the biochemically active molecules to be bound.

7. A support according to any one of claims 1 to 6, incorporating a spatially varying pattern (18) for identification purposes.

8. A support according to claim 7, wherein said pattern is a barcode.

9. A support according to claim 8, wherein the barcode is a linear barcode.

10. A support according to any one of claims 7 to 9, in which the pattern comprises a series of holes (2) in the support.

11. A method of fabricating the supports of one of claims 1 to 10, comprising sputter coating a flat surface with a metal layer (13), anodising the metal layer, and lithographically patterning and etching the metal layer to reveal the supports.

12. A method according to claim 13, wherein said surface consists of a layer of soluble material (12) on a rigid substrate (11), and the method further comprises releasing the supports from said surface by solvation of the soluble material.

13. A method according to claim 12, wherein the soluble material is a resist.

14. A method according to any one of claims 11 to 13, wherein the anodising is carried out at a voltage of up to 150 V.

15. A method according to claim 14, wherein the anodising is carried out at a voltage in the range from 4 V to 30V.

16. A method according to any one of claims 11 to 15, further comprising binding probe molecules (16) to the anodised metal layer.

17. An optical reader for reading the patterns and identifying the supports according to claim 7.

18. A reader according to claim 17, operating by means of transmission optics.

19. A reader according to claim 18, wherein said supports are transported through an optical read volume by a fluidic system.

20. A reader according to claim 18, in which there are

two substantially orthogonal light transmission paths (33, 34).

21. A reader according to claim 20, incorporating one or more fluorescence detectors.

FIGURES

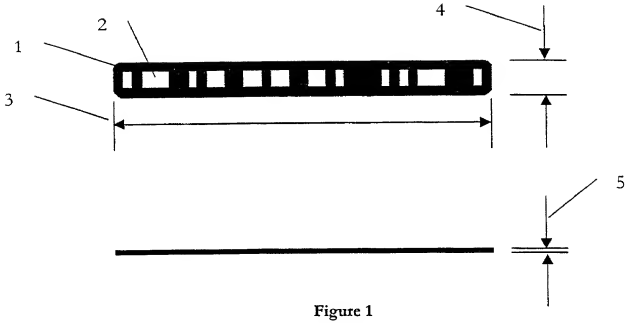

Figure 1

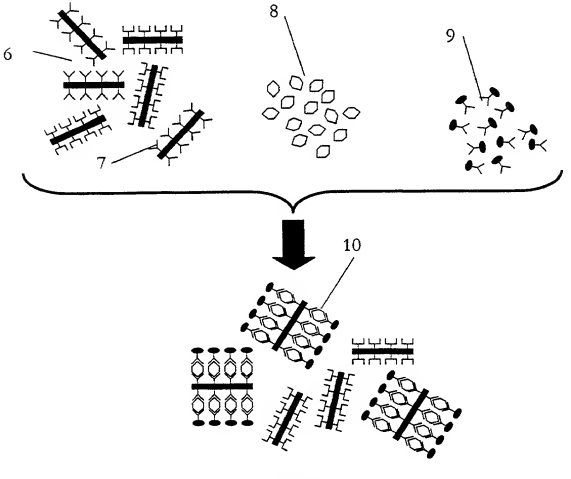

Figure 2

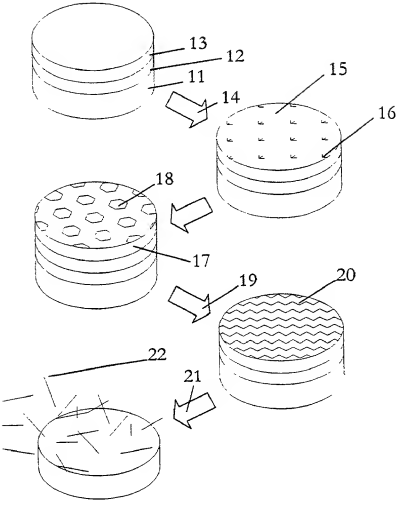

Figure 3

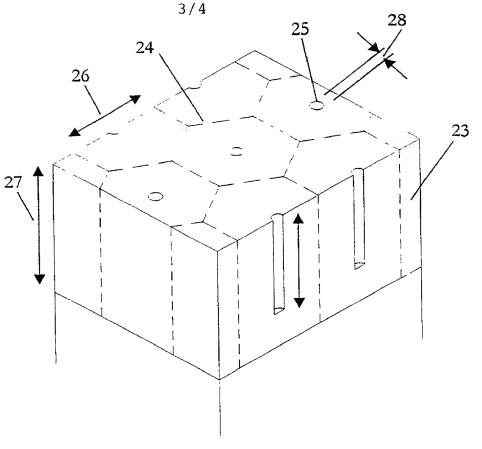

Figure 4

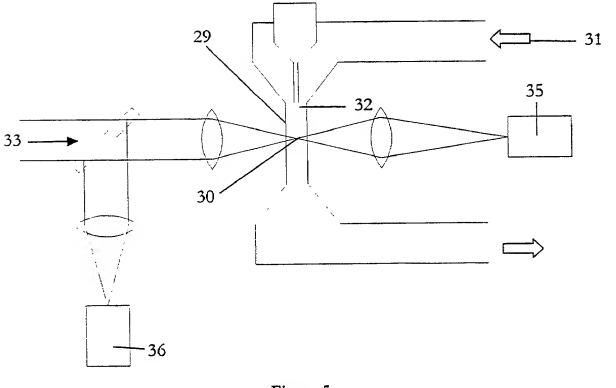

Figure 5

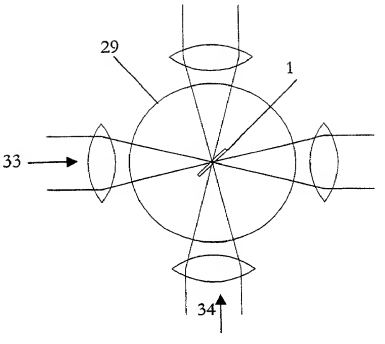

Figure 6

DECLARATION FOR USA PATENT APPLICATION
(including Design and National Stage PCT)

Attorney's Docket ID: _____

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below adjacent to my name.
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention

entitled BIO-ASSAY TECHNIQUE

_____, the specification of which

(check one)

_____ is attached hereto

_____ is U.S. Application Serial No. _____ which was filed on _____

and (if applicable) amended on _____

X Is International Application No. PCT/GB99/03109 which was filed on 17 SEPTEMBER 1999

and (if applicable) amended on 7 NOVEMBER 2000

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.
I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).
I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s) (__ ADDITIONAL APPLICATIONS IDENTIFIED ON ATTACHED SHEET):

Number	Country	Day/Month/Year Filed	Priority Yes	Claimed No
9820163.5	GB	17 SEPTEMBER 1998	X	

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below; and insofar as the subject matter of each claim of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application in accordance with 37 C.F.R. 1.63(d) (___ ADDITIONAL APPLICATIONS IDENTIFIED ON ATTACHED SHEET).

Application Serial No.	Day/Month/Year Filed	Status -- patented, pending, abandoned

I hereby appoint the following to prosecute and transact all business in the Patent and Trademark Office connected therewith:

Andrew E. Taylor - 1700	Thomas P. Sarro - 19396	Douglas E. Jackson - 28618	Harold L. Novick - 26011
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Marvin Petry - 22752	William E. Jackson - 24016	Linda R. Poteate - 36255	

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Facsimile Tel: (703) 892-8428

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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SIGN AND DATE HERE	Inventor's Signature	Date	5/3/2001
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SIGN AND DATE HERE	Inventor's Signature	Date	5/3/2001

X SEE ATTACHED SHEET FOR SIMILAR INFORMATION AND SIGNATURE FOR ADDITIONAL JOINT INVENTORS.

Declaration, Power of Attorney and Power to Inspect

Inventors:

3-00

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